EXHIBIT A

ERYTHROPOIETIN, NITRIC OXIDE SYNTHASE AND RESISTANCE TO MYOCARDIAL ISCHEMIA

Rabbits adapted to chronic hypoxia exhibit increased resistance to myocardial ischemia, resulting from increased nitric oxide production from endothelial nitric oxide synthase (1). However, the sensor responsible for detecting hypoxia resulting in increased nitric oxide production is unknown. The adequacy of renal tissue oxygenation at Epo-producing sites regulates Epo production (2), but a more potent extrarenal oxygen sensor may exist (3). L-NAME partially blocks increase in plasma levels of Epo in mice following exposure to hypoxia, thus implicating nitric oxide in oxygen sensing and Epo production (4). Epo directly stimulates atrial natriuretic peptide secretion from adult rat atria but not cultured myocyte (5). These data suggest Epo may play a role in adaptation of hearts to chronic hypoxia and resistance to ischemia by a NOS related mechanism.

Hypothesis 1: **Chronic hypoxia results in increased Epo production that subsequently controls nitric oxide production from NOS.**

1. Measure Epo receptors in normoxic and hypoxic hearts.
   Availability of antibody to Epo

Hypothesis 2: **Epo increases nitric oxide production from NOS3.**

2. Treat normoxic rabbits acutely with Epo, is there an increase in nitric oxide production resulting in cardioprotection.

References

1. Shi Y, Pritchard Jr., KA, Holman P, Rafiee P, Griffith OW, Kalyanaraman B, Baker JE. Chronic myocardial hypoxia increases nitric oxide synthase and decreases caveolin-3. Free Radic Biol Med 29:695-703, 2000.

2. Kurtz A, Eckardt KU. Renal function and oxygen sensing. In: *Erythropoietin: Molecular, Cellular, and Clinical Biology*, edited by A.J. Erslev, J.W. Adamson, J.W. Eschbach, and C.G. Wineari. Baltimore, MD: Johns Hopkins University Press, 1991, P. 79-98.

3. Pagel H, Jelkmann W, Weiss C. O_2 supply to the kidneys and the production of erythropoietin. Respir Physiol 77:111-118, 1989.

4. Ohigashi T, Brookins J, Fisher JW. Interaction of nitric oxide and cyclic guanosine 3',5'-monophosphate in erythropoietin production. J Clin Invest 92:1587-1591, 1993.

5. Porat O, Neumann D, Zamir O, Nachshon S, Feigin E, Cohen J, Zamir N. Erythropoietin stimulates atrial natriuretic peptide secretion from adult rat cardiac atrium. J Pharmacol Exp Ther 276:1162-1168, 1996.

John E. Baker, Ph.D.
[redacted] 2001

β Gal

EPO 5units/ml treatment for 24 hrs

IP eNOS

IB

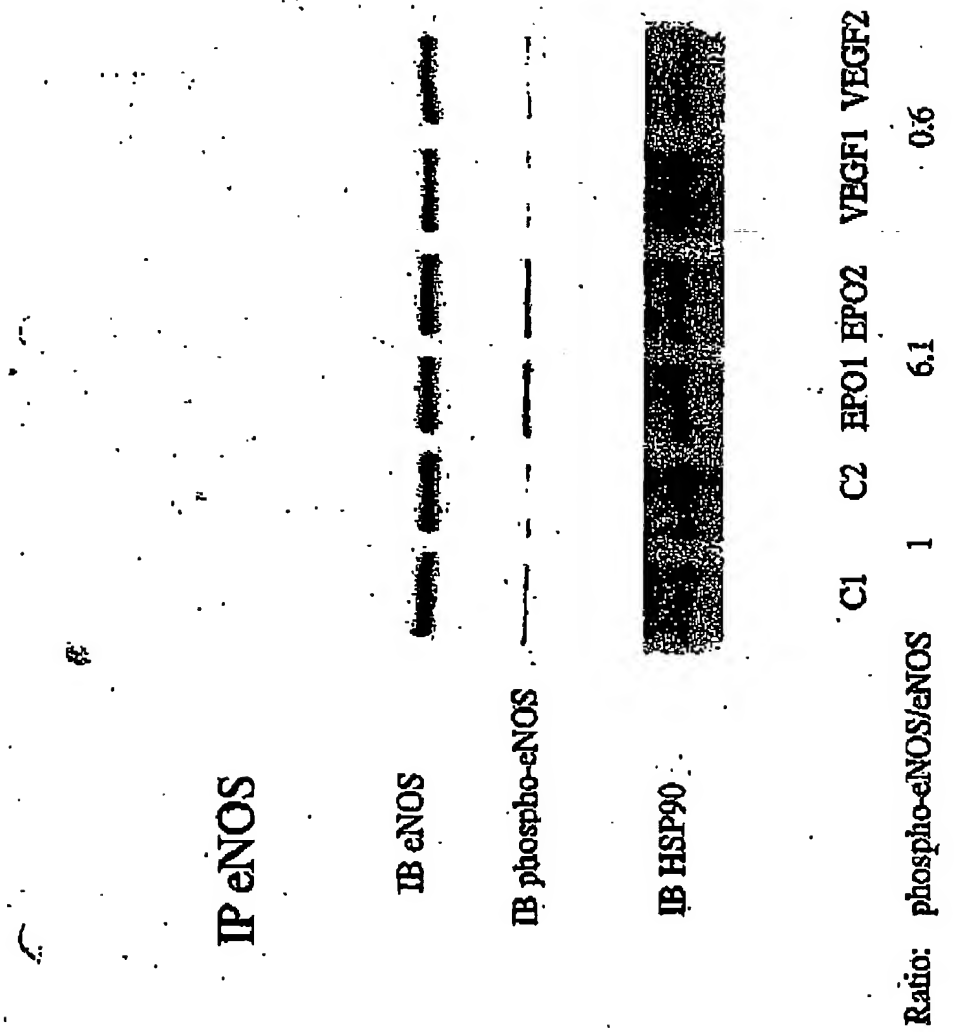
Phospho-eNOS

IB

Hsp90

C1 C2 EPO1 EPO2 VEGF1 VEGF2

+ve control



Date: [redacted], 2002

MCW Research Foundation
Discovery Record and Report

EXHIBIT B

1. Brief descriptive title: Cardioprotection by Erythropoietin
2. Full name of discoverer(s), home address(es), and position(s):
   a. John E. Baker, Ph.D., 2131 N. 72 St., Wauwatosa, WI 53213 Professor
   b. Yang Shi, Ph.D., 2116 N. 115 St., Wauwatosa, WI 53226 Post doctoral fellow
   c.
3. Results to be achieved by the practice of this discovery:
   Improved resistance of the heart to ischemia.
4. Brief description of the discovery: (Attach additional pages of description if necessary).
   See attachment
5. Chronology of conception and reduction to practice:
   a. Date of earliest conception: [redacted]
   b. Date of disclosure (orally or in writing) to other persons and names of such persons: [redacted]
   c. First written record pertinent to discovery: [redacted]
   d. Date and result of first test of the discovery: [redacted]
6. Source, number and size of grant(s) used to support the research relating to this discovery:
   Departmental funding and NIH HL54075 $[redacted]
7. Date and place of publication or anticipated publication: (Attach copy of publication if available.)
   Autumn 2002
8. List any published information on known practices in the field of the discovery which is pertinent:

Witness: Mary Lynne Seeg

Discoverer: J.E. Baker
Name: John E. Baker, Ph.D. Date [redacted] 2002

Yang Shi
Name: Yang Shi, Ph.D. Date [redacted] 2002

Name: Date

4. Brief description of the discovery

Erythropoietin is a key blood glycoprotein that initiates and regulates red blood cell production. Erythropoietin is approved by the FDA for human use in the treatment of anemia. We determined if erythropoietin can increase the resistance of the heart to ischemia. Hearts from New Zealand White rabbits were perfused with erythropoietin (0.5 – 10.0 U/ml) for 15 min prior to a global ischemic insult of 30 min followed by 35 min reperfusion. Erythropoietin exhibited a dose-dependent cardioprotective effect with optimal cardioprotection observed at 1.0 U erythropoietin/ml. Cardioprotection was manifest by a highly significant increase in recovery of pre-ischemic left ventricular developed pressure from 48±3% to 75±4%. We believe this is the first demonstration of cardioprotection by erythropoietin.